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Research paper

Internal validation of the RapidHIT® ID system

Rachel Wiley[a,*], Kelly Sage[a], Bobby LaRue[b], Bruce Budowle[a,c]

[a] Center for Human Identification, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, TX, 76107, United States
[b] Sam Houston State University, Huntsville, TX, 77340, United States
[c] Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia

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ABSTRACT

Traditionally, forensic DNA analysis has required highly skilled forensic geneticists in a dedicated laboratory to generate short tandem repeat (STR) profiles. STR profiles are routinely used either to associate or exclude potential donors of forensic biological evidence. The typing of forensic reference samples has become more demanding, especially with the requirement in some jurisdictions to DNA profile arrestees. The Rapid DNA (RDNA) platform, the RapidHIT® ID (IntegenX®, Pleasanton, CA), is a fully automated system capable of processing reference samples in approximately 90 min with minimal human intervention. Thus, the RapidHIT ID instrument can be deployed to non-laboratory environments (e.g., booking stations) and run by trained atypical personnel such as law enforcement. In order to implement the RapidHIT ID platform, validation studies are needed to define the performance and limitations of the system. Internal validation studies were undertaken with four early-production RapidHIT ID units. Reliable and concordant STR profiles were obtained from reference buccal swabs. Throughout the study, no contamination was observed. The overall first-pass success rate with an "expert-like system" was 72%, which is comparable to another current RDNA platform commercially available. The system's second-pass success rate (involving manual interpretation on first-pass inconclusive results) increased to 90%. Inhibitors (i.e., coffee, smoking tobacco, and chewing tobacco) did not appear to affect typing by the instrument system; however, substrate (i.e., swab type) did impact typing success. Additionally, one desirable feature not available with other Rapid systems is that in the event of a system failed run, a swab can be recovered and subsequently re-analyzed in a new sample cartridge. Therefore, rarely should additional sampling or swab consumption be necessary. The RapidHIT ID system is a robust and reliable tool capable of generating complete STR profiles within the forensic DNA typing laboratory or with proper training in decentralized environments by non-laboratory personnel.

1. Introduction

Traditionally, forensic DNA typing has been performed by highly skilled analysts in a dedicated laboratory environment to obtain a short tandem repeat (STR) profile(s). STR typing is used to associate or exclude individuals as potential contributors of forensic biological evidence. Even with current capabilities, the processes can be laborious and time-consuming. Simply, there are not enough resources, including trained laboratory personnel, available to analyze the samples currently being submitted to forensic laboratories for DNA testing. In addition, the delay introduced from the time of collection to generation of a genetic profile can be substantial and has been attributed to the early release of suspects, particularly in the case of arrestees, who are potentially associated with previous crimes. Rapid DNA (RDNA) platforms can help address some of the challenges confronting the forensic laboratory as well as investigators. The instruments are fully automated, integrated systems capable of generating STR profiles from reference samples in less than 90 min with little hands-on requirements [1–9].

The aim of using RDNA systems is to provide the community with a tool that is both robust and capable of being operated within the laboratory or by non-laboratory personnel in decentralized environments such as booking stations, small-scale laboratories, national security agencies, border security patrol, etc. Even though these systems could be operated in decentralized environments, they should still be managed and supervised from a regulatory forensic body [1,3,6]. Current RDNA platforms are limited in that they require multiple samples to be run in parallel. First-generation RDNA systems were not ideal for these decentralized environments, primarily because a set of samples had to be run simultaneously for the analysis to be cost effective. Therefore, a sufficient number of samples need to be collected and then run which could impact the immediate or real-time analysis of reference swabs collected. The RapidHIT® ID (IntegenX®, Pleasanton, CA) is a second

* Corresponding author at: University of North Texas Health Science Center, 3500 Camp Bowie Blvd, Fort Worth, TX, 76107-2699, United States.
E-mail address: Rachel.Wiley@my.unthsc.edu (R. Wiley).

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generation, compact (less than 28 cm wide) RDNA instrument that is capable of single sample analysis. The system is fully automated and integrated for generating a STR profile with minimal hands on requirements in approximately 90 min (initial "hands-on" time is less than one minute for swab loading). With this system, law enforcement or other personnel could collect a reference buccal sample outside of the traditional laboratory setting and generate a Combined DNA Index System (CODIS) uploadable profile which could be searched against a DNA database for real-time development of investigative leads and prior to a person of interest's release from custody [3,5,8]. This accelerated process could prevent early release of potential criminal offenders associated with previous crimes, consequently, decreasing the potential for future crimes and thwarting a criminal's attempt to escape jurisdiction prior to formal investigation [10]. Additionally, in moving the processing of routine arrestee samples out of the forensic laboratory and into decentralized environments, the demand placed on current forensic scientists could be lessened, allowing their focus to be on the more challenging analysis of forensic biological evidence that does require their knowledge and expertise.

An internal validation of the RapidHIT ID system, applicable to reference samples and modified RDNA analysis, was conducted in accordance with the Scientific Working Group on DNA Analysis Methods (SWGDAM) issued internal validation guidelines [11]. Modified RDNA analysis refers to platforms that are fully automated for the development of STR genetic profiles; however, they require manual interpretation of the genetic data to confirm genotype determination [4,5]. The RapidHIT ID system was evaluated for overall genotyping success, contamination, reliability, sensitivity, inhibition, mixture detection, swab stability, precision, swab substrate type, and sample re-analysis. The concordance study additionally was used to examine the system's metrics such as average peak height and heterozygosity balance. Herein the RapidHIT ID platform's performance was assessed in the role as an expert-like system for STR genotype reporting.

2. Materials and methods

The RapidHIT ID system incorporates into a single integrated unit DNA extraction, direct amplification of specific STR loci via the polymerase chain reaction (PCR), separation of the PCR products by electrophoresis, and analysis of the genetic data [3,6]. The multiplex of GlobalFiler® Express (Thermo Fisher Scientific, Waltham, MA), an approved National DNA Index System (NDIS) chemistry, is typed within the instrument system [3,12]. The single-use, consumable RapidHIT ID sample cartridge contains the GlobalFiler® Express primer set and master mix preloaded in separate reagent vials (Fig. 1). The sizing standard is preloaded into an additional sample cartridge reagent vial. Because of the length of time dedicated to this study, different sample cartridge and primary cartridge lots were used. Once a STR profile is produced, the instrument transfers the data to a central computer for viewing in GeneMarker® HID (SoftGenetics, State College, PA). Control cartridges, preloaded with an allelic ladder (IntegenX), were used to type the samples [3].

2.1. Sample collection

Buccal swab samples from unrelated individuals were collected using 6 inch Puritan® Sterile Cotton Tipped Applicators (Puritan, Guilford, ME) and were anonymized. This swab type was used for most studies with the exception of the substrate study where multiple swab types were tested. For the concordance study, 50 unique individuals were tested. Three different individuals were included for the completion of all other studies. All samples were collected in accordance with the approved protocols set forth by the Institutional Review Board for the University of North Texas Health Science Center (UNTHSC) in Fort Worth, TX. Samples were stored at room temperature until use. Depending on the study, samples were run on one to four RapidHIT ID instruments. The number of instruments used is stated in each study description. A validation plan outlining the specific features of each study is depicted in Supplemental Table 1.

2.2. Contamination assessment study

Run-to-run contamination was assessed by analyzing buccal swabs and blanks in an alternating pattern on four instruments as illustrated in Supplemental Table 2. The sample and blank profiles generated were evaluated for evidence of contamination between runs.

2.3. Reliability study

For the reliability study, four different individuals were analyzed in a checkerboard pattern on two RapidHIT ID instruments (Supplemental Table 3). Each contributor was typed a total of four times to determine whether each sample consistently produced the same corresponding genetic profile.

2.4. Swab re-analysis study

To examine the potential for swab recovery of a failed run and subsequent re-analysis on the RapidHIT ID system, 12 buccal swabs were run. Re-analysis refers to the same swab being removed from one sample cartridge and placed into a new cartridge in order to be typed an additional time. For this study, at the completion of the first run, the same sample swabs were removed from their cartridges and placed into new sample cartridges for re-analysis. This process was performed for a third run on two RapidHIT ID instruments (six buccal swabs on each system). Each buccal swab was run a total of three times (total study n = 36).

2.5. Sensitivity study

A series of dilutions were prepared from neat saliva (average 4.88 ± 0.35 ng/μL of DNA, quantified in triplicate) from a single contributor with a known reference profile. Neat saliva was diluted using molecular biology grade water (Phenix, Candler, NC). Dilutions prepared from neat saliva were used as a more controlled approach to examine the system's sensitivity parameters than an alternative method employing "cheek swipes" where the amount of DNA introduced with each sampling can potentially vary. DNA was extracted using an EZ1® DNA Investigator Kit (Qiagen, Hilden, Germany) following the "Pretreatment for Forensic Surface and Contact Swabs" protocol and the "DNA Purification (Trace Protocol)" described in the manufacturer's manual. Determination of the quantity of DNA was performed using the Qubit™ Fluorometer (Thermo Fisher Scientific, Waltham, MA) and the Qubit™ dsDNA BR Assay Kit (Thermo Fisher Scientific, Waltham, MA). Initially, a broad dilution series was tested (Supplemental Table 4A) which was followed by a narrower dilution series (Supplemental Table 4B). The neat saliva was agitated for resuspension of the cells prior to sample aliquoting for each dilution within the two series; however, the individual cells transferred were not counted. Each swab was prepared by pipetting 10 μL of the respective dilution onto the tip of a Puritan® Sterile Cotton Tipped Applicator, allowed to air dry for approximately 20 min, and subsequently placed into the sample chamber of a consumable cartridge. Triplicates of each dilution were typed to assess threshold signals on two RapidHIT ID instruments.

2.6. Inhibitor study

The inhibitor study consisted of two samples from five individuals for each inhibitor who had either consumed coffee, chewed (smokeless) tobacco, or smoked tobacco within 5 min prior to collection (total study n = 30). These samples were typed across all four RapidHIT ID instruments. Baseline reference samples for all inhibitor study

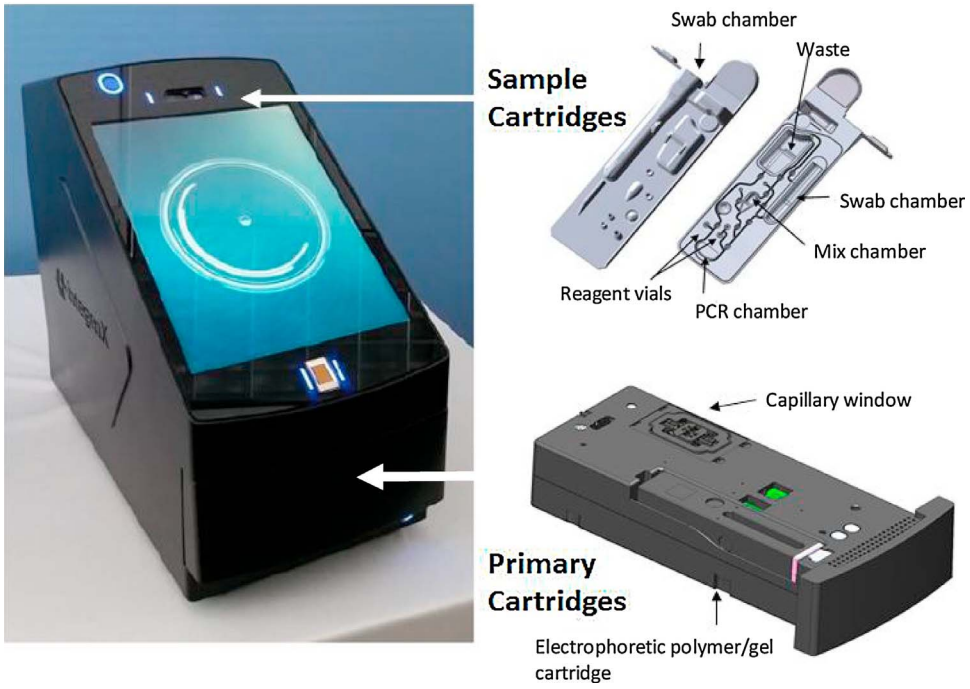

Fig. 1. RapidHIT ID instrument (images provided by IntegenX) for Rapid DNA analysis with labeled sample and primary cartridges (IntegenX personal communication) [3].

contributors were collected prior to inhibitor exposure and were compared for concordance.

2.7. Mixture study

Although not for the intended application, a limited mixture study was conducted to evaluate whether mixture profiles would be flagged under the RapidHIT ID system conditions. Mixed-source samples, consisting of saliva from two contributors at approximately a ratio of 1:1, were analyzed. Couples were asked to have one individual initially swab with the second individual swabbing immediately after with the same buccal swab. Additionally, mixture samples were prepared manually from one couple at a ratio of 1:9 (vol:vol).

2.8. Swab stability study

The swab stability study involved testing of swabs from 0 to 6 months of age from six different donors. Due to sample availability, a different contributor was sampled at each time point. Baseline profiles were generated for each contributor immediately after collection (i.e., 0 month time interval).

2.9. Precision study

Ten GlobalFiler® Express Kit ladder cartridges were run consecutively on one instrument for the precision study.

2.10. Concordance study

Buccal swabs from 50 participants were collected for the concordance study. Three Puritan® Sterile Cotton Tipped Applicators were collected from each person and one of these swabs was typed via traditional capillary electrophoresis (CE) methods, another swab was subjected to RDNA, and the third swab was maintained as a back-up if needed. For the swabs subjected to CE analysis, DNA was extracted using a Qiagen QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) following the "Buccal Swab Spin Protocol" described in the manufacturer's manual. The quantity of extracted DNA was determined using the Quantifiler® Human DNA Quantification Kit (Thermo Fisher Scientific, Waltham, MA) on an Applied Biosystems® 7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA) following the manufacturer's protocol. Amplification of 2 ng DNA was performed using the GlobalFiler® Express PCR Amplification Kit (Thermo Fisher Scientific, Waltham, MA) on the GeneAmp PCR System 9700 (Thermo Fisher Scientific, Waltham, MA). The PCR products were separated on an Applied Biosystems™ 3500 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA). Electropherograms were analyzed with GeneMapper® Software v5 (Thermo Fisher Scientific, Waltham, MA). Genotypes were exported in the CODIS v3.2 format for upload into the IntegenX reference database for concordance comparison. This database was accessed by the RapidMatch software program (IntegenX). RapidMatch compares RapidHIT ID profiles to the known genotypes entered in the database and identifies any inconsistencies between genotypes, denotes any flags present within the generated profiles, and includes specific run metrics.

The second swab from each study participant was used to generate a STR profile on the RapidHIT ID platform. Electropherograms were processed and analyzed through GeneMarker® HID v2.8.8 Software (SoftGenetics). All profiles generated on the RapidHIT ID system were manually checked for concordance with their CE-generated counterparts. Additionally, the genetic profiles produced by the RapidHIT ID system were evaluated using RapidMatch.

2.11. Swab substrate study

Buccal swab samples were collected using Puritan® Sterile Cotton Tipped Applicators and anonymized for the majority of the studies conducted. The following collection devices were tested on the RapidHIT ID system to determine the versatility of the instrument: 4N6-FLOQSwabs™ (Copan Flock Technologies, Bresica, Italy), MacroPur™ Swabs (Solon Manufacturing Company, Rhinelander, WI), and 3 mm punches from Whatman FTA cards (GE Healthcare, Waltham, MA). The EasiCollect (GE Healthcare, Waltham, MA) also was examined including the swab itself and two 3 mm punches of the corresponding sample card from the same donor. All buccal samples were collected by performing three swipes with the designated swab on each cheek of the oral cavity for a total of six swipes per swab, with the exception of the Whatman FTA cards and the EasiCollect. For the Whatman FTA cards,

Table 1
Summary of results for all studies conducted for the internal validation of the RapidHIT ID system.

Study Description	Sample Type	Number of Total Runs	Number of First-Pass Successful Runs	Number of Second-Pass Successful Runs	Number of Failed Runs (Instrument/ Cartridge)	Number of Re-Analysis Runs	Number of Re-Analysis Successful Runs
Contamination Assessment Study	Blank Cotton Swab	16	13	16	0	0	0
	Buccal on Cotton Swab	17	12	16	1	1	1
Reliability Study	Buccal on Cotton Swab	16	11	14	2	2	2
Swab Re-Analysis Study	Buccal on Cotton Swab (First Run)	12	8	11	1	0	0
	Buccal on Cotton Swab (Second Run)	12	9	10	2	0	0
	Buccal on Cotton Swab (Third Run)	12	7	8	4	0	0
Sensitivity Study	Broad Dilution Series	30	0	12	18*	0	0
	Narrow Dilution Series	24	0	3	21*	0	0
Inhibitor Study	Coffee	10	9	10	0	0	0
	Chewing Tobacco	11	8	9	1	1	1
	Smoking Tobacco	10	9	10	0	0	0
Mixture Study	1:1 Mixture	10	N/A	N/A	N/A	N/A	N/A
	1:9 Mixture	1	N/A	N/A	N/A	N/A	N/A
Swab Stability Study	Buccal on Cotton Swab	6	6	6	0	0	0
Precision Study	GlobalFiler Express Allelic Ladder	10	10	10	0	0	0
Concordance and Reliability Studies	Buccal on Cotton Swab	60	41	49	10	10	10
Swab Substrate Study	4N6-FLOQSwabs	11	4	10	1	1	1
	MacroPur	9	5	8	1	1	1
	FTA 3 mm Punch	4	0	4	0	0	0
	EasiCollect Swab	8	0	4	4	0	0
	EasiCollect 3 mm Punch	8	0	8	0	0	0
	FTA 3 mm Punch (2 punches)	5	1	4	0	0	0
Standards/Control Study	NIST Standard Pipetted onto FTA punch	3	0	3	0	0	0
	1000 M Swab	3	3	3	0	0	0
Bridge Study	Buccal on Cotton Swab	20	18	20	0	0	0

* These runs were counted under the failed run category; however, the results are likely due to low level sample since they were part of the sensitivity study.

saliva samples were collected on Puritan® Sterile Cotton Tipped Applicators and then transferred to the FTA card by the rolling/pressing application method with sample margins marked. Sample collection for EasiCollect samples followed the manufacturer's protocol. Two contributors were examined on all four instruments for each swab substrate type. All samples were allowed to air dry prior to typing and were stored at room temperature in paper packaging.

2.12. Standards/Control study

The NIST standard SRM® 2391c PCR-Based DNA Profiling Standard was evaluated on the RapidHIT ID platform. Components A, B, and C consist of single-source genomic DNA with concentrations ranging from 1.1-2.1 ng/μL. Component A is from a single source female, whereas components B and C are from single-source male donors [13]. An aliquot of 10 μL of the respective NIST standard components (A through C) was pipetted onto two pre-punched 3 mm FTA punches placed within a microcentrifuge tube and allowed to air dry. Once dried, the two punches were loaded into the swab chamber of their corresponding sample cartridge for typing on the RapidHIT ID system following the manufacturer's protocol for testing sample standards (IntegenX personal communication).

Additionally, swabs with the 1000 M reference cell line (HTB-157) were examined on the RapidHIT ID system for comparison with the results obtained in the developmental validation by IntegenX [3,14]. IntegenX provided swabs consisting of 50,000 1000 M cells spotted on 3 inch Puritan® Sterile Cotton Tipped Applicators. Three 1000 M swabs were run on the RapidHIT ID platform to determine whether the results were concordant.

2.13. Bridge study

Due to updates on the RapidHIT ID system during the validation study, a small bridge study (n = 20) was conducted. This study was to assess if the overall typing capability was concordant and reproducible regardless of software modifications.

2.14. Data analysis

The data obtained in the concordance study also were used to determine performance metrics. Data analysis used the default flag parameters established by IntegenX for the RapidHIT ID system during its developmental validation [3]. First-pass success was defined as the system's ability to generate complete and concordant genetic profiles with no profiles or alleles flagged by the analysis software. Flags were assigned when the system's stringent standards were not met on a per locus basis (e.g., analytical and/or stochastic thresholds were not met or heterozygous peaks were notably imbalanced). A second-pass success

was described as any run profiles, not captured in the first-pass analysis, which would be classified as complete following manual interpretation. A run would be identified as a failure if the resulting run profile either was not generated or a partial profile was produced, which could be attributed to instrument and/or cartridge failure, or too little DNA on the swab.

Traditional CE profiles were analyzed by GeneMapper® Software v5, and RapidHIT ID electropherograms were processed by Trace Analyzer v2016.0906.1 (Trace Analyzer v2016.0906.1 (IntegenX), Santa Clara, CA) and analyzed through GeneMarker® HID v2.8.8 Software. RapidMatch was employed to assess concordance between the RapidHIT ID system and conventional DNA analysis methods. Any sample that was not typed due to instrumentation or cartridge failure was retyped to show that the sample was not lost should a failure occur. Similar to the re-analysis study, any swab that did not type initially was removed from the cartridge and placed in a secondary sample cartridge for re-analysis. In only a few instances was a sample required to be rerun, meaning that a new swab was analyzed on that RapidHIT ID platform.

3. Results and discussion

3.1. Contamination assessment study

There were no instances of contamination observed on all instruments evaluated. Artifacts were present in three of the 16 blank swabs tested (Table 1 and Supplemental Fig. 1). None of the peaks observed in these blank samples corresponded to the sample profiles run in this study. In two of these instances, some peaks fell within allele bins; however, in both profiles, the observed peaks were consistent throughout the dye channels supporting that these peaks are artifacts and not due to carry over contamination. The third blank profile showed a single artifact peak that did not fall within an allele bin. The samples typed were concordant with reference profiles generated with traditional CE methods.

3.2. Reliability study

Fourteen of the 16 samples typed for the reliability study yielded the same respective profile for each of the four contributors (Table 1). Instrument/cartridge failure was observed in two of the 16 runs in this study; however, when the same swabs for these samples were re-analyzed (i.e., same swab, new cartridge), the profiles generated were complete and concordant.

3.3. Swab re-analysis study

The swab recovery and re-analysis study produced concordant genetic profiles with minimal allelic dropout observed in select profiles (Table 1). All alleles attributed to dropout were present, but below threshold and therefore were not called by the RapidHIT ID system. There was one instance of non-concordance (an allele was called at the DYS391 locus); however, this locus was manually reviewed and found to be the result of pull-up. There were 12 sample recovery sets run on two RapidHIT ID instruments (6 sets on each system). Each recovery set consisted of one swab being analyzed three times. As previously described, a sample would be typed on the RapidHIT ID and following the completion of that run, the swab would be removed from the cartridge and placed into a new cartridge for analysis. This swab re-analysis was performed a third time (total study n = 36). Four of the 12 recovery sets run indicated an instrument/cartridge failure for one of the triplicate runs with one set consisting of all three replicate runs failing. This sample set did not yield any results. The genetic data for these runs failed to transfer to the central computer for analysis and interpretation. When a run failure occurred, the same sample swab was removed from its current sample cartridge and was placed in a new sample cartridge for re-analysis. In each sample recovery set, a first-pass success complete profile was generated for at least one run or for all runs. First-pass success was achieved for 24 of 36 runs (67% success) with second-pass success (after manual interpretation) including 29 of the 36 runs (81% success). The remaining runs experienced instrumentation/cartridge failure.

When using the RapidHIT ID instrument, whether performing an initial analysis or re-analysis, one must always be cognizant about the potential of contamination. For re-analysis of the same swab, amplified product potentially contaminating the sample swab is unlikely because the swab is never exposed to amplified product. A small portion of the sample DNA is removed from the swab, sent through the sample cartridge to another separate chamber for amplification. Therefore, standard handling methods should be sufficient regarding controlling for contamination.

3.4. Sensitivity study

This RDNA platform was designed for analysis of high quality DNA (i.e., reference samples). Sensitivity studies are not particularly relevant for this application; however, they were conducted for illustrative purposes regarding the system's capacity to generate complete STR profiles. Both a broad and narrow dilution series were conducted on two RapidHIT ID systems. Triplicates were tested for each dilution to determine sensitivity of detection under the conditions applied in this study (Table 1). The samples consisting of a 1:9 dilution generated complete STR profiles; however, the 1:19 dilutions had substantial allelic dropout. These results were consistent between the two RapidHIT ID instruments. The dilution series showed that the conservative limit of detection to be the 1:9 dilution for the two instruments on which this study was performed.

3.5. Inhibitor study

All potential inhibitors (coffee, chewed tobacco, and smoked tobacco) had minimal to no effect on genotyping (Table 1). No allelic dropout was observed. Two profiles (one smoking tobacco and one chewing tobacco) of the 30 tested swabs, showed instances of imbalanced heterozygous peaks, but all peaks were appropriately called. Additionally, one coffee inhibitor profile showed atypical peak morphology at the D19S433 locus resulting in the locus being flagged for manual review. The D8S1179 locus in an adjacent dye channel also depicted migration issues during separation as shown by a flagged signal indicating its position, denoted as "OB", was out of established GlobalFiler® Express allele bins (Supplemental Fig. 2A). When the separation current data were examined, there appeared to be a drop in the current being applied to the capillary which could be attributed to the migration issues observed at these two loci (Supplemental Fig. 2B). Unusual peak morphology should indicate a need to examine additional data associated with the run including the separation current data and perhaps perform swab re-analysis prior to forming a final conclusion. Supplemental Fig. 2C shows the separation current data for a successful RapidHIT ID system run.

The average peak heights (expressed in Relative Fluorescence Units, or RFUs) for the three inhibitors are shown in Fig. 2. Chewing tobacco tainted samples had the highest average peak height (1995.31 ± 1053.58 RFUs) followed by coffee tainted samples (1947.97 ± 884.24 RFUs) and with smoking tobacco tainted samples depicting the lowest signal (1367.60 ± 653.69 RFUs). The average peak heights for the samples with inhibitors were comparable to the average peak heights for the reference baseline profiles obtained prior to inhibitor exposure (1721.30 ± 811.25 RFUs).

3.6. Mixture study

Each profile generated from a 1:1 mixed-source sample was flagged

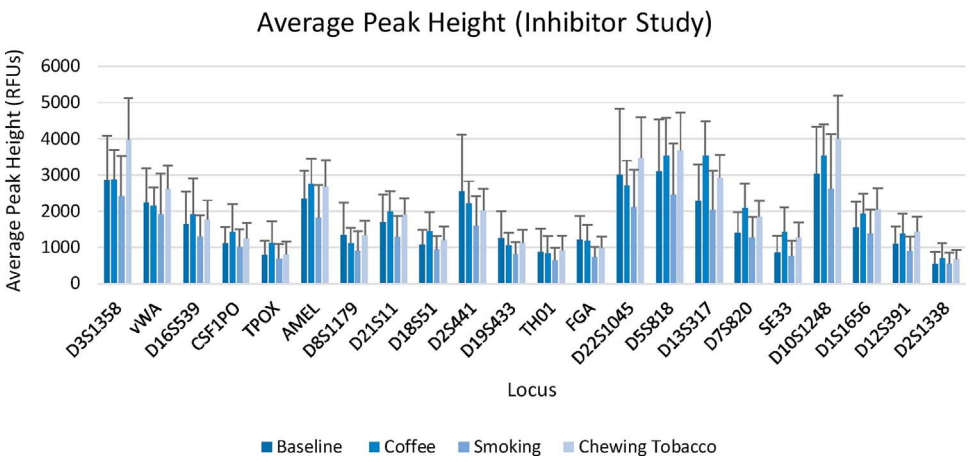

Fig. 2. Average peak heights (RFUs) observed for samples analyzed in the inhibition study. Each inhibitor consisted of two samples from five individuals (total study n = 30). The average peak heights for the baseline (untainted) reference samples were 1721.30 ± 811.25 RFUs. The average peak heights for coffee were 1947.97 ± 884.24 RFUs, smoking tobacco were 1367.60 ± 653.69 RFUs, and chewing tobacco were 1995.31 ± 1053.58 RFUs.

by the RapidHIT ID system. Although prepared as a 1:1 mixture, the profiles presented with a major and minor contributor in each mixture, which is likely due to different cell content in the samples and/or the manner in which the mixture samples were collected. The second contributor was typically the major contributor and thus may have removed a portion of the epithelial cells from the initial donor. For the 1:9 mixture sample, manually prepared, the profile was concordant with the major contributor's profile with the exception of a single allele above the analytical threshold at a small locus (D3S1358) for which the minor contributor was homozygous. Since the number of epithelial cells suspended can be variable from sample-to-sample and dilution-to-dilution, the results for the mixture study reported herein are relative and are only for illustrative purposes on performance.

3.7. Swab stability study

While the RapidHIT ID system was designed for fresh reference swabs, the effect of sample swab age on genetic typing success was assessed. All samples typed (from 0 to 6 months of age) for the swab stability study yielded complete profiles on the first-pass review (i.e., no alleles flagged) indicating that the DNA could be extracted from the swabs as well as there was not substantial degradation over time (Table 1). All samples were concordant with their respective fresh reference baseline profiles.

3.8. Precision study

The allelic ladder was run 10 times consecutively to assess precision of measurements. The results of the precision study are displayed in Fig. 3. The highest size standard deviation recorded in this precision study sample set was 0.084 bases. Even at three standard deviations (0.252 bases), the allele variance is well within accepted tolerances. Similar precision results were reported in the RapidHIT ID system's developmental validation study [3].

3.9. Concordance study

All buccal swabs typed were concordant with profiles generated by traditional CE methods. A total of 60 runs was required to complete the RDNA concordance and reliability study, meaning that, of the 50 concordance participants studied, a portion of the swabs required re-analysis and/or rerunning (Table 1). The overall first-pass success rate was 72% (36 of the 50 concordance samples), which is comparable to the DNAScan/ANDE™ Rapid DNA Analysis™ platform (Network Biosystems, Waltham, MA) performance reported by Moreno et al. [8]. Flags are generated to identify potential typing issues and are designed to cast a wide net. Thus, flags tend to err on the side of caution and will identify results at times that are interpretable and reliable. For example, GeneMarker® HID Software places saturation flags on any peaks that migrate at the same position, but in adjacent dye channels (IntegenX personal communication). Two of the concordance sample runs had these described flags. Additionally, one sample contained multiple flags for "OB" signals. This sample had minus A peaks throughout the profile that were called "OB". The large number of minus A peaks were likely due to sample overload. Finally, one profile contained flags for heterozygous peak imbalance. The sister allele was 32% of the parent allele; however, the peak was also the same size as an allele in an adjacent dye channel. The flagged allele was concordant, but due to its peak height and positioning, it was classified as inconclusive. The system's second-pass success rate (involving manual interpretation of first-pass flagged results) increased to 90% (45 of the 50 concordance samples), which exceeded the performance reported (at best 77.5% success with the inclusion of partial profiles and those where peak imbalance was observed) for the DNAScan/ANDE™ Rapid DNA Analysis™ platform [8].

There was an 8% instrument and/or cartridge failure rate (4 of the 50 samples failed). These samples required the same swab to be re-analyzed or the remaining swab from initial collection to be typed for a second time on the RapidHIT ID system.

For all of the first-pass success runs, the average peak height for each locus is shown in Fig. 4. The average peak heights indicated generally a decrease (or sloping effect) as the locus size increased in each of the dye channels. Peak height ratios for the same sample set are illustrated in Fig. 5. Average peak height ratios for all heterozygous peaks in all concordance samples (n = 50) were 0.85 ± 0.03.

Only one sample (RHID49) was unable to produce a complete profile following multiple attempts at re-analysis and a secondary swab being typed on the RapidHIT ID system. Allelic dropout was observed in every run at the SE33 locus for this sample. The contributor is heterozygous at the SE33 locus with alleles 18 and 26.2 (as determined by conventional DNA analysis methods). Allele 26.2 is present in each run; however, its peak height is below the system's established analytical threshold for the SE33 locus and was not called. The SE33 locus is a large amplicon, and allele dropout is more likely than for other loci which can be exacerbated if the swab contains too little DNA for analysis. The peak heights at each locus in this sample were below the average peak heights across all samples studied for concordance.

3.10. Swab substrate study

The Puritan® Sterile Cotton Tipped Applicators were used throughout the validation study, and thus, were demonstrated to be compatible to the RapidHIT ID system. Other collection materials were tested for compatibility with the system. Swab substrate study results are summarized in Table 1. Generally, the 4N6-FLOQSwab™ samples and the MacroPur™ swabs performed better than the other materials tested. The FTA punches and two different samplings from the

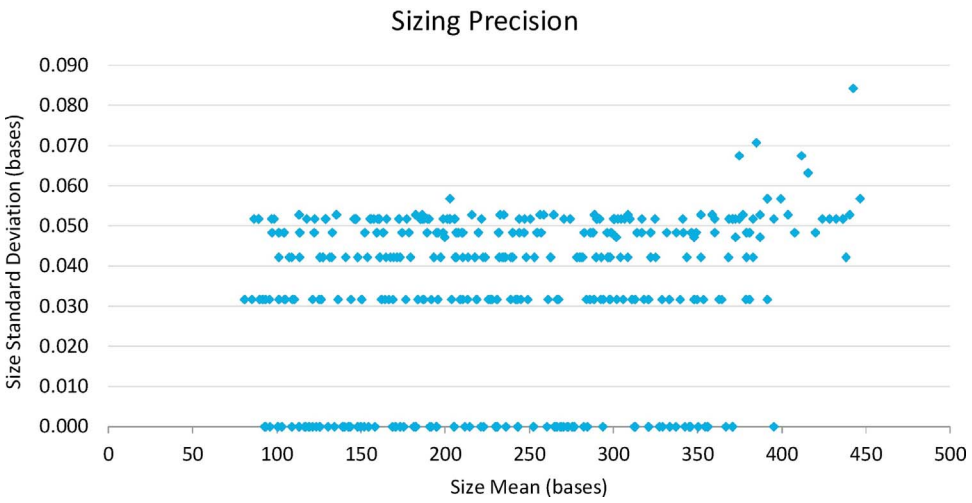

Fig. 3. Size precision data of 10 GlobalFiler® Express ladders run on one instrument.

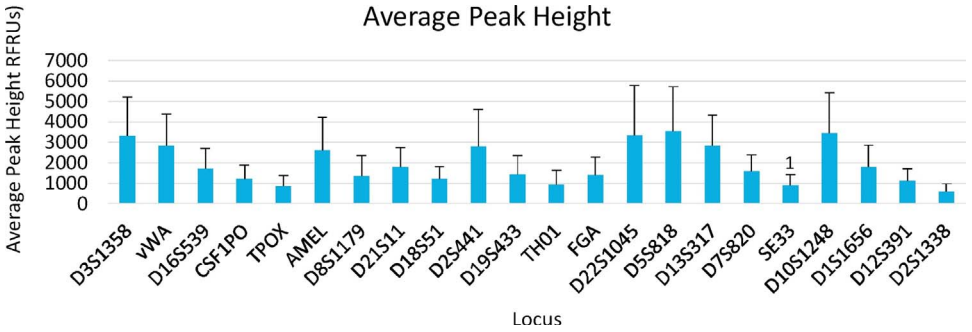

Fig. 4. Average peak heights (RFUs) observed for all samples yielding a complete profile typed in the concordance study (n = 50) for all GlobalFiler® Express loci (minus Y-indel and DYS391 loci).
[1]Sample RHID49 was the only sample that consistently experienced dropout at the SE33 locus. Both heterozygous alleles were present, but one was below threshold and was not called. The average height of the two alleles for RHID49 was manually calculated and included in the height calculation for the SE33 locus.

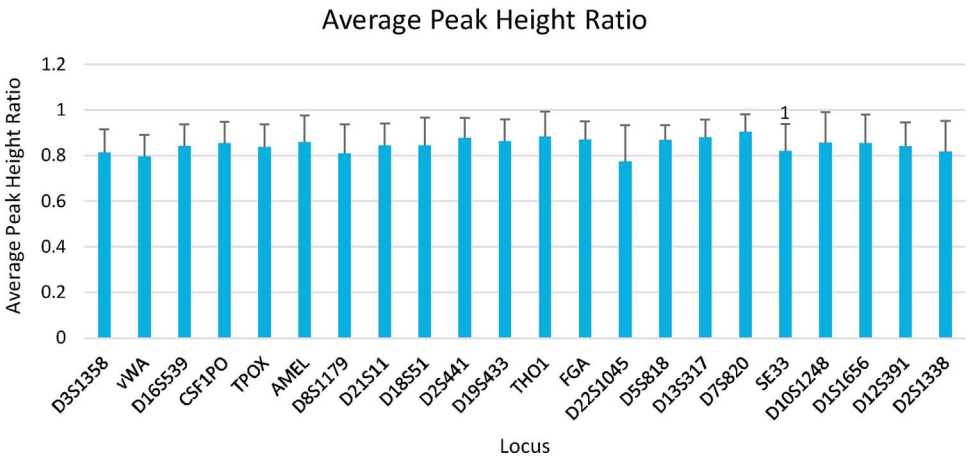

Fig. 5. Average peak height ratios for all GlobalFiler® Express loci for the same sample set of concordance samples as shown in Fig. 4.
[1]Since sample RHID49 failed to call both heterozygous peaks at the SE33 locus; the sample's SE33 peak height ratio measurement was calculated manually and included in Fig. 5.

EasiCollect device (3 mm punches from card and swab) performed poorly overall by yielding only partial or blank profiles. The addition of a second FTA punch was tested. In one instance, a full profile was generated; however, in all other runs, only partial profiles were produced with low signal intensity. All called alleles for the substrate study were concordant with the reference profiles produced by CE. The results for these collection materials should be considered preliminary; more studies should be performed to perfect sample collection and analysis within the RapidHIT ID system with various collection devices.

3.11. NIST standard study

NIST standard SRM® 2391c PCR-Based DNA Profiling Standard components A, B, and C all experienced minimal allelic dropout (i.e., no more than four alleles dropping out per sample) according to the threshold parameters previously established by the RapidHIT ID system [3]. The alleles experiencing "dropout" were all present, but below the threshold for allele calling (Table 2). The low quantity of NIST standard components that were tested is likely the cause of the incomplete profiles being generated. The signal of the NIST standards pipetted onto the two 3 mm pre-punched FTA punches was consistent with the 1:9 to 1:19 dilution (5.4 ng and 2.6 ng, respectively) and substrate type samples described above where allelic dropout was observed. Complete profiles were generated in triplicate for the 1000 M cell line (HTB-157) impregnated swabs (Table 2). These swabs contained 50,000 cells spotted onto a single swab which is a far greater amount of DNA than can be typed using the NIST standards. Each 1000 M replicate was concordant with the genotype reported within the NIST standard Certificate of Analysis for component F (HTB-157) [13,14].

Table 2
Allele calls made by the RapidHIT ID system for the NIST standard study including the testing on components A through C and 1000 M spotted swabs [13,14]. Blue boxes denote observed allelic dropout (first-pass evaluation).

Locus	NIST Standard A	RapidHIT ID Standard A	NIST Standard B	RapidHIT ID Standard B	NIST Standard C	RapidHIT ID Standard C	NIST Standard F	RapidHIT ID 1000M
D1S1656	17.3,17.3	17.3	11,14	11,14	11,15	11,15	17.3,17.3	17.3,17.3
D2S1338	18,23	18,23	17,17	17,17	19,19	19,19	17,17	17,17
D2S441	10,10	10,10	10,14	10,14	10,10	10,10	14,14	14,14
D3S1358	15,16	15,16	15,19	15,19	16,18	16,18	16,17	16,17
D5S818	11,12	11,12	12,13	12,13	10,11	10,11	11,13	11,13
D7S820	11,11	11,11	10,10	10,10	10,12	12	8,12	8,12
D8S1179	13,14	13,14	10,13	10,13	10,17	10,17	10,13	10,13
D10S1248	15,16	15,16	13,13	13,13	12,16	16	14,15	14,15
D12S391	18.3,22	18.3,22	19,24	19,24	19,23	19,23	18,19	18,19
D13S317	8,8	8,8	9,12	9,12	11,11	11,11	8,11	8,11
D16S539	10,11	10,11	10,13	10,13	10,10	10,10	9,11	9,11
D18S51	12,15	12,15	13,16	13,16	16,19	16,19	17,22	17,22
D19S433	13,14	13	16,16.2	16,16.2	13.2,15.2	13.2,15.2	13,14	13,14
D21S11	28,32.2	32.2	32,32.2	32,32.2	29,30	29	29,32.2	29,32.2
D22S1045	15,15	15,15	15,17	15,17	16,16	16,16	11,15	11,15
CSF1PO	10,10	10,10	10,11	10,11	10,12	10,12	10,11	10,11
FGA	21,23	21	20,23	20,23	24,26	24,26	21,25	21,25
SE33	16,18	16,18	17,18	17,18	28.2,31.2	28.2,31.2	12,21	12,21
THO1	8,9.3	8,9.3	6,9.3	6,9.3	6,8	6,8	7,9.3	7,9.3
TPOX	8,8	8,8	8,11	8,11	11,11	11,11	8,8	8,8
vWA	18,19	18,19	17,18	17,18	16,18	16,18	16,18	16,18
Amelogenin	X,X	X,X	X,Y	X,X	X,Y	X,X	X,Y	X,Y
DYS391			10	10	11	11	12	12
Y-Indel				2		2	2	

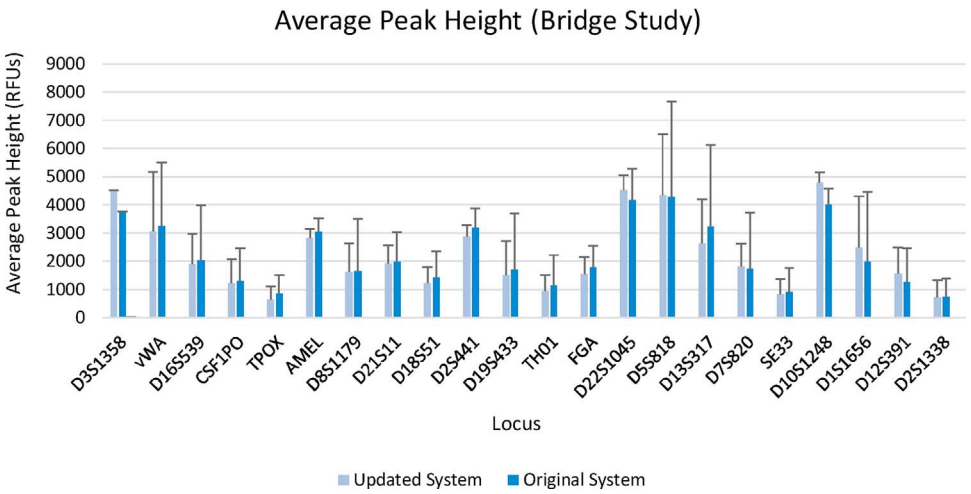

Fig. 6. Average peak height (RFUs) observed for all samples typed for the bridge study (n = 20). Ten samples were run both on the initial and current iteration of the RapidHIT ID system. The average peak height for the updated system was 2247.62 ± 1305.93 RFUs while the average for the original system was 2245.91 ± 1152.92 RFUs.

3.12. Bridge study

All samples within the bridge study sample set (n = 20; 10 from initial runs and 10 from final runs) generated concordant genetic profiles on the updated RapidHIT ID system with those originally produced with earlier version software (Fig. 6). The performance is consistent between the runs with the initial sample set yielding an average peak height of 2245.91 ± 1152.92 RFUs and the updated system runs generating an average peak height of 2247.62 ± 1305.93 RFUs.

4. Conclusions

The RapidHIT ID instrument was validated in accordance with the SWGDAM recommended guidelines [11]. Throughout the validation studies, no contamination was observed and reproducible genetic profiles were generated. Although some of the studies (i.e., mixture and sensitivity studies) were not applicable to the intended use of the RapidHIT ID system, these studies were conducted to better understand the performance and operation of the instrument. The system was able to detect mixed-source samples and, in the event of a failed run, sufficient sample remained on the swab for recovery and re-analysis. Inhibitors and swab age did not appear to influence typing. In contrast, sample collection substrate varied in performance; however, these other collection devices were not optimized for the instrument. The overall findings of this study indicate that the RapidHIT ID system is a reliable tool for the typing of reference samples.

Conflict of interest

The authors declare to have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fsigen.2017.09.011.

References

[1] S. Gold, RapidDNA: a game changer in the law enforcement identification stakes, Biom. Technol. Today 2013 (1) (2013) 7–10.
[2] B.L. LaRue, A. Moore, J.L. King, P.L. Marshall, B. Budowle, An evaluation of the RapidHIT® system for reliably genotyping reference samples, Forensic Sci. Int. Genet. 13 (2014) 104–111.
[3] S. Salceda, A. Barican, J. Buscaino, B. Goldman, J. Klevenberg, M. Kuhn, D. Lehto, F. Lin, P. Nguyen, C. Park, F. Pearson, R. Pittaro, S. Salodkar, R. Schueren, C. Smith, C. Troup, D. Tsou, M. Vangbo, J. Wunderle, D. King, Validation of a rapid DNA process with the RapidHIT® ID system using GlobalFiler® Express chemistry, a platform optimized for decentralized testing environments, Forensic Sci. Int. Genet. 28 (2017) 21–34.
[4] M. Date-Chong, W.R. Hudlow, M.R. Buoncristiani, Evaluation of the RapidHIT™ 200 and RapidHIT GlobalFiler® Express kit for fully automated STR genotyping, Forensic Sci. Int. Genet. 23 (2016) 1–8.
[5] FBI, Rapid DNA Analysis, Available at https://www.fbi.gov/services/laboratory/biometric-analysis/codis/rapid-dna-analysis.
[6] M. Holland, F. Wendt, Evaluation of the RapidHIT™ 200, an automated human identification system for STR analysis of single source samples, Forensic Sci. Int. Genet. 14 (2015) 76–85.
[7] H.S. Mogensen, R. Frank-Hansen, B.T. Simonsen, N. Morling, Performance of the RapidHIT™ 200, Forensic Sci. Int. Genet. Suppl. Ser. 4 (1) (2013) e286–e287.
[8] L.I. Moreno, A.L. Brown, T.F. Callaghan, Internal validation of the DNAscan/ANDE™ Rapid DNA Analysis™ platform and its associated PowerPlex® 16 high content DNA biochip cassette for use as an expert system with reference buccal swabs, Forensic Sci. Int. Genet. 29 (2017) 100–108.
[9] S. Verheij, L. Clarisse, M. van den Berge, T. Sijen, RapidHIT™ 200, a promising system for rapid DNA analysis, Forensic Sci. Int. Genet. Suppl. Ser. 4 (1) (2013) e254–e255.
[10] Z. Thong, Y.H. Phua, E.S. Loo, B.H. Shue, C.K.C. Syn, Investigative leads from DNA: Casework experience from the IntegenX RapidHIT™ 200 System, Forensic Sci. Int. Genet. Suppl. Ser. 5 (2015) e69–e70.
[11] Scientific Working Group on DNA Analysis Methods (SWGDAM), Validation Guidelines for DNA Analysis Methods, (Approved December 2012) Available at https://www.swgdam.org.
[12] L.K. Hennessy, N. Mehendale, K. Chear, S. Jovanovich, S. Williams, C. Park, S. Gangano, Developmental validation of the GlobalFiler® express kit a 24-marker STR assay, on the RapidHIT® System, Forensic Sci. Int. Genet. 13 (2014) 247–258.
[13] National Institute of Standards and Technology, Certificate of Analysis: Standard Reference Material 2391 PCR-Based DNA Profiling Standard, in: D.o. Commerce (Ed.) Gaithersburg, MD, 2015.
[14] M.C. Kline, C.R. Hill, E.L. Butts, D.L. Duewer, M.D. Coble, J.M. Butler, Examination of DNA Mixture Proportion Variability Using Multiple STR Typing Kits and NIST Standard Reference Material® 2391 Component D, (2011).